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Association of ectopic fat with abdominal aorto-illiac and coronary artery calcification in african ancestry men

Allison L. Kuipers [a, *], Joseph M. Zmuda [a], J. Jeffrey Carr [b], James G. Terry [b], Sangeeta Nair [b], Ryan Cvejkus [a], Clareann H. Bunker [a], Alan L. Patrick [c, 1], Christina L. Wassel [d], Iva Miljkovic [a]

[a] Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA
[b] Department of Radiology, Vanderbilt University Medical Center, Nashville, TN, USA
[c] Tobago Health Studies Office, Scarborough, Tobago, Trinidad & Tobago
[d] Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT, USA

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ABSTRACT

Background and aims: There is strong evidence that fat accumulating in non-adipose sites, "ectopic fat", is associated with cardiovascular disease (CVD), including vascular calcification. Most previous studies of this association have assessed only a single ectopic fat depot. Therefore, our aim was to assess the association of total, regional, and ectopic fat with abdominal aorto-illiac calcification (AAC) and coronary artery calcification (CAC) in 798 African ancestry men.

Methods: Participants (mean age 62) were from the Tobago Bone Health Study cohort. Adiposity was assessed via clinical examination, dual x-ray absorptiometry, and computed tomography (CT). Ectopic fat depots included: abdominal visceral adipose tissue (VAT), liver attenuation, and calf intermuscular adipose tissue (IMAT). Vascular calcification was assessed by CT and quantified as present *versus* absent. Associations were tested using multiple logistic regression adjusted for traditional cardiovascular risk factors. Models of ectopic fat were additionally adjusted for total body fat and standing height.

Results: All adiposity measures, except VAT, were associated with AAC. Lower liver attenuation or greater calf IMAT was associated with 1.2–1.3-fold increased odds of AAC ($p < 0.03$ for both), though calf IMAT was a stronger predictor than liver attenuation ($p < 0.001$) when entered in a single model. No ectopic fat measure was associated with CAC.

Conclusions: Greater adiposity in the skeletal muscle and liver, but not in the visceral compartment, was associated with increased odds of AAC in African ancestry men. These results highlight the potential importance of both quantity and location of adiposity accumulation throughout the body.

1. Introduction

There is strong evidence that fat accumulating in non-adipose sites, "ectopic fat", is associated with cardiometabolic disease, including cardiovascular disease (CVD; [1]). While there have been previous studies of the association between ectopic adipose tissue and vascular calcification [2–23], a marker of subclinical CVD, many studies assessed only a single depot. The existing research has also primarily focused on Caucasians and may not be applicable to other races because of known racial/ethnic differences in body composition [24,25] and cardiovascular risk [26]. Specifically, African ancestry men are known to be generally lean, but have higher cardiovascular disease risk compared to Caucasians [26]. We hypothesize that differences in ectopic fat distribution may be one explanation for this apparent paradox. Indeed, we recently reported that intermuscular adipose tissue (IMAT) in the calf muscle, is associated with increased risk of diabetes [27], hypertension [28], and mortality [29], independent of total and central adiposity in African ancestry men. Therefore, we hypothesize that IMAT may be an ectopic fat depot of particular importance for vascular calcification, as well, in African ancestry men. In the current study, we

* Corresponding author. Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, 130 DeSoto Street, A543 Crabtree Hall, Pittsburgh, PA 15261, USA.
E-mail address: kuipers@pitt.edu (A.L. Kuipers).
[1] Deceased.

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tested if whole body, regional, and ectopic fat measures were associated with vascular calcification in the coronary and abdominal aorto-illiac arteries in a sample of 798 African ancestry men.

2. Materials and methods

2.1. Tobago Health Study

All men in this analysis were from the Tobago Bone Health Study (TBHS), a population-based, prospective cohort study of community-dwelling men aged 40 years and older, residing on the Caribbean island of Tobago [30]. Participants in the TBHS were recruited without regard to health status and men were eligible if they were ambulatory, not terminally ill and without a bilateral hip replacement. Men from Tobago are of homogeneous African ancestry with low European admixture (<6%) [31]. The 798 men for the current analysis were recruited consecutively at follow-up visits completed between 2011 and 2016, which consisted of chest, abdominal and calf computed tomography (CT) scans, total body dual x-ray absorptiometry (DXA), clinical examination and interviewer-administered health history questionnaires. Written informed consent was obtained from each participant using forms and procedures approved by the University of Pittsburgh Institutional Review Board, the U.S. Surgeon General's Human Use Review Board, and the Tobago Division of Health and Social Services Institutional Review Board.

2.2. Vascular calcification

Vascular calcification was assessed by chest and central CT using a dual slice, high-speed NX/I scanner (GE Medical Systems, Waukesha, WI). The scans were obtained using the axial, two-slice scan mode (2i) and a segmented (a.k.a "half-scan") reconstruction to provide an effective temporal resolution of approximately 350 msec for each 3 mm thick slice without cardiac gating. Coronary artery calcification (CAC) values were obtained from cross-sectional slices through the chest from the carina through the entire inferior aspect of the heart and measurements made by vessel for each of the major epicardial coronary arteries. For the abdominal scan series, a helical scan mode (120 KVp, 250 mA, 3 mm slice thickness and pitch of 1.5:1) was utilized since the higher temporal resolution for the coronary arteries was not required. Aortic artery calcification (AAC) values were obtained from cross-sectional slices through the abdomen from L3 to S1 and included the summation of calcification in the abdominal aorta and common iliac arteries. Measurements were performed by experienced analysts using an FDA approved computer workstation and software (Calcium, Aquarius workstation, TeraRecon San Mateo, CA) that accounts for slice thickness and scan field of view. The Agatston method [32] was used to report scores of calcified plaque. The lead reader for this study also led the Coronary Artery Risk Development In young Adults (CARDIA) Study CT analyses, and a blinded re-read of 153 CARDIA scans found intra-reader technical error of 6.6%.

For the current analysis, an Agatston score >10 defined presence of CAC or AAC to further reduce false positive classification [33]. We also calculated the Agatston to volume ratio (AVR), which is a measure of calcified plaque density that takes into account both the total volume of plaque and the amount of calcification, using the recent methods described by Bellasi et al. [34].

2.3. Adiposity assessment

Adiposity was measured using clinical examination (body weight, body mass index (BMI), and waist circumference), whole body DXA (total percent fat and percent fat in the trunk), abdominal CT [abdominal subcutaneous adipose tissue (SAT), abdominal visceral adipose tissue (VAT) and liver attenuation], and peripheral quantitative CT [calf intermuscular adipose tissue (IMAT)]. Body weight was measured to the nearest 0.1 kg on a balance beam scale and standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, both without participants wearing shoes. Body mass index was calculated as body weight in kilograms divided by standing height in meters squared. Waist circumference was measured at the level of the umbilicus or greatest circumference using a flexible tape measure. Total body fat percent and percent fat in the trunk were measured via DXA using a Hologic QDR 4500 W densitometer (Hologic, Bedford, MA).

Abdominal adiposity was assessed using the CT scans collected to measure vascular calcification as discussed above. Measures of abdominal SAT and VAT were obtained from the same cross-sectional images that were used for AAC assessment from L3 to S1. Liver attenuation was assessed in Hounsfield units (HU) from 3 contiguous scan slices taken in the T12 to L1 space. Calf IMAT was measured via peripheral quantitative CT performed with a Stratec XCT-2000 scanner (Orthometrix, Inc.; White Plains, NY). A site at 66% of the calf length, proximal to the terminal end of the tibia was scanned, since it has the largest circumference and the lowest variability in composition between individuals [35]. All images were analyzed with STRATEC analysis software version 5.5D (Orthometrix, Inc., White Plains, NY) and performed by a trained investigator who was unaware of the participant's vascular calcification status. For this analysis, ectopic fat was assessed using abdominal VAT, liver attenuation and calf IMAT; whereas, all other measures were considered to be measures of general or regional adiposity.

2.4. Other characteristics

Demographic, health history and anthropomorphic characteristics were assessed by trained staff via interview and clinical exams. Blood pressure was measured three times while seated and the average of the 2nd and 3rd reading were used in this analysis. Hypertension was defined as a systolic blood pressure (SBP) $\geq$140 mmHg, diastolic blood pressure (DBP) $\geq$90 mmHg, current self-reported use of antihypertensive medication or an affirmative to the question "has a doctor ever told you that you have hypertension or high blood pressure?" Fasting blood was drawn at the time of interview and was spun into serum samples and aliquoted and frozen at −80 °C on the day of blood draw. Fasting serum glucose was measured using an enzymatic procedure and fasting serum insulin was measured using a radioimmunoassay procedure developed by Linco Research, Inc. Glucose is presented in mg/dL units that can be converted to SI units by dividing by 18. Insulin is presented in μU/mL units that can be converted to SI units by multiplying by 6.945. The degree of insulin resistance was estimated by homeostasis model assessment (HOMA-IR) according to the method described by Matthews et al. [36]. Diabetes was defined as a fasting serum glucose level $\geq$126 mg/dL when fasting measures were available (N = 711), and/or current self-reported use of diabetes medication, or an affirmative response to the question "has a doctor ever told you that you have diabetes?" Serum high-density lipoprotein cholesterol (HDL-c) was determined using the selective heparin/manganese chloride precipitation method. Serum low-density lipoprotein cholesterol (LDL-c) was calculated by means of the Friedewald equation. Triglycerides were determined enzymatically using the procedure of Bucolo and David [37]. Dyslipidemia was defined as having HDL-c <40 mg/dL or triglycerides >150 mg/dL when fasting measures were available (N = 686), and/or current self-reported use of statins or other lipid-lowering medications.